

## Effect of Parenteral Liver Extract on the Blood Picture of New Born Rats.\*

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Previous work<sup>1, 2, 3</sup> has demonstrated the essential similarity in the transition toward normal adult levels of the peripheral blood pictures of new born rats and of the human with macrocytic hyperchromic anemia treated with liver extract. The transition in both may be interpreted<sup>3, 4, 5</sup> as the result of the substitution of normoblastic erythropoiesis in the place of megaloblastic erythropoiesis. There is, therefore, the possibility that the administration of liver extract may accelerate this transition in new born rats.

A control series of observations on the blood picture changes in 288 normal rats from birth to 24 days of age has been compiled to serve as a basis of evaluation of the effect of such an experimental procedure (see Table 3, reference 3).

The degree of variability or scatter as expressed by the standard deviation is proportionally large and markedly broadens the limits of non-significance ( $X \pm 3\sigma$ ). Inasmuch as the young do not grow at the same rate, it is to be expected that blood formation in the different individuals will proceed at different rates. Litter mates frequently showed as large variations and differences as among unrelated animals of the same age. Data on the state of hemoconcentration in these rats are not available. A larger daily population would have smoothed the curves and reduced the variations.

Because of these considerations it is emphasized that only means, which are not too sharply delineated, and trends may safely be recognized. A sample of the error of observation is indicated by the coefficients of variation derived from 10 determinations on one blood specimen (see Table I, reference 3).

Two approaches have been utilized in determining the effect of

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<sup>1</sup> Wintrobe, M. M., and Shumacher, H. B., *J. Clin. Invest.*, 1935, **14**, 837.

<sup>2</sup> Smith, C., *J. Path. and Bact.*, 1932, **35**, 717.

<sup>3</sup> Bruner, H. D., Van de Erve, J., and Carlson, A. J., *Am. J. Physiol.*, 1938, **124**, 620.

<sup>4</sup> Jones, O. P., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **38**, 222.

<sup>5</sup> Kato, K., *J. Lab. and Clin. Med.*, 1935, **20**, 1243.

liver extract on the blood picture of young rats: (I) The injection of liver extract into the mother rats before and during pregnancy, with examination of the blood of the young at intervals after birth. (II) The injection of liver extract directly into the young after birth, with examination of the blood at intervals after injection.

The liver extract used was a clinically active commercial extract (several different batches) formerly designated as 3 cc derived from 100 g of liver, and now as 10 units per 3 cc.† The rats used were of the same stock and fed the same diets as the control rats; further, most of the observations were carried out during the same period. The technical and hematological methods were as previously described<sup>3</sup> and frequent checks were made to assure that the error of observation remained within fixed limits.

(I) Liver extract in doses of 2.5 cc per kg body weight was injected intramuscularly into 19 female rats once per week for 1 to 4 weeks before mating and throughout pregnancy. This weekly quantity of extract was estimated, on a weight basis, to be approximately 10 to 15 times the total quantity required to induce remission in the average case of pernicious anemia.

The blood values of the 144 young of these injected rats were determined in 32 rats within 24 hours after birth and in 14 young on the third day after birth and every third day thereafter up to the twenty-fourth day of age. The young of a litter were distributed over the entire period as far as possible.

The means and the standard deviations (Table I) of the blood values grouped as to age were checked against the control data by the method of the standard error of the difference of two means. The mean values of the erythrocytes, hemoglobin, and hematocrit were not significantly different from the corresponding means except in the instance of the erythrocytes on the twelfth, fifteenth, and eighteenth days; the means of the hemoglobin and hematocrit also tended to be higher on these days. In view of the definite non-significance on the ninth and twenty-first days, the differences do not assume importance. The increased values might readily be explained by hemoconcentration. The mean values of the mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration were not significantly different. The mean values of the reticulocytes, percent and absolute, were significantly different from the means of the control groups only on the sixth day. It will be noted that the control value for this day is out of line. The leucocyte values

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TABLE I.  
Means (X) and Standard Deviations (σ) of Blood Values of 144 Young Rats from Liver Extract Injected Mothers.

Age in Days	N*	R.B.C.*		Hb.*		Ht.*		M.C.V.*		M.C.Hb.*		M.C.Hb.C.*		%		Absolute		W.B.C.*	
		X	σ	X	σ	X	σ	X	σ	X	σ	X	σ	X	σ	X	σ	X	σ
1	32	2.560	0.354	11.56	1.50	36.49	5.92	143.9	14.7	45.8	5.7	31.7	2.3	92.6	4.7	2.369	0.360	4.43	1.92
3	14	2.525	0.460	9.29	0.71	31.12	3.00	124.0	10.7	37.2	4.3	30.6	3.2	46.1	12.4	1.238	0.422	3.57	1.08
6	14	2.530	0.254	8.20	0.40	28.79	1.14	114.5	9.2	32.7	2.6	28.5	1.5	42.7	11.1	1.047	0.151	3.58	1.39
9	14	3.175	0.320	8.93	0.81	30.71	2.78	97.3	5.4	23.2	3.2	29.0	2.3	38.6	8.8	1.242	0.238	3.84	0.93
12	14	3.745	0.342	8.72	0.81	29.73	2.63	80.0	5.8	23.4	2.0	29.3	1.8	26.1	5.5	0.956	0.199	4.13	0.73
15	14	4.315	0.411	8.92	0.76	30.00	3.05	69.9	5.7	20.8	1.3	29.7	1.5	21.0	5.0	0.903	0.217	4.25	1.87
18	14	4.575	0.597	8.41	1.10	27.70	4.31	61.4	7.1	18.5	3.2	30.5	1.7	20.4	4.4	0.922	0.205	4.34	0.93
21	14	4.715	0.565	8.26	0.83	26.68	2.32	57.2	5.2	17.7	2.2	31.0	1.4	20.4	7.0	0.978	0.466	4.41	1.00
24	14	5.140	0.425	9.36	1.27	31.79	4.56	62.0	6.8	18.2	2.2	29.5	1.6	23.2	6.7	1.190	0.627	3.95	1.07

\*N = Number of rats; R.B.C. = Erythrocytes in millions per mm<sup>3</sup>; Hb. = Hemoglobin in g per 100 cc blood; Ht. = % packed erythrocytes; M.C.V. = Mean corpuscular volume in cubic micra; M.C.Hb. = Mean corpuscular hemoglobin in gamma gamma; M.C.Hb.C. = Mean corpuscular hemoglobin concentration in %; Reticulocytes expressed in % erythrocytes and absolute in millions per mm<sup>3</sup>; W.B.C. = Leucocytes in thousands per mm<sup>3</sup>.

showed no significant differences. Only 1.2% of the 1,306 measurements were beyond the limits of the control group; 0.8% of the measurements were also located beyond the limit of the  $X \pm 3\sigma$  for the injected series.

There was found, thus, a lack of consistent differences between the examined blood values of the experimental and control groups. While it is possible that some of the young of the injected mothers showed significant differences, the majority did not. Further, rather large differences were sometimes obtained between similar blood values of litter mates of the same age as in the control group.

It may be added that the injection of liver extract did not prevent the appearance, qualitatively, of the prepartum anemia of the mother rat as described by Mitchell and Miller<sup>6</sup> and by Van Donk, Feldman and Steenbock.<sup>7</sup>

(II) The liver extract used for injecting the young rats was diluted 5 times with distilled water and injected subcutaneously. Leakage was prevented by sealing the puncture with a stiff vaseline. The dose used was 5 cc per kg body weight of undiluted extract and was calculated to be approximately 20 to 25 times the remission inducing dose for the human pernicious anemia patient. A sterile saline solution containing 0.5% phenol was used for control injections.

Twenty-eight litters each of 8 or more young were used. For each 2 young rats injected with diluted liver extract, 1 litter mate was injected with salt solution to serve as control. Two litters were injected within 8 hours after birth, and similarly 2 litter groups were injected for each day after birth up to 14 days of age. Studies were made on 2 liver extract-injected rats and 1 control rat of the litter on the fourth, tenth, fifteenth and, if the litter were large enough, twenty-first days after injection, recording the blood values according to age. Of the 182 liver extract-injected young, 30 were examined after the twenty-fourth day and are not included. Since the values of the young of one age at the different intervals after injection are distributed indiscriminately among one another, the means and standard deviations of the whole distribution are presented in Table II.

The means of the erythrocyte values of the injected rats from the twelfth to the eighteenth days tended to be higher than the means of the controls but the difference was not significant nor was the in-

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<sup>6</sup> Mitchell, H. S., and Miller, L., *Am. J. Physiol.*, 1931, **98**, 311.

<sup>7</sup> Van Donk, E. C., Feldman, H., and Steenbock, H., *Am. J. Physiol.*, 1934, **107**, 1934.

TABLE II.  
Means (X) and Standard Deviations (σ) of Blood Values of 152 Liver Extract Injected Young Rats.

Age in Days	N*	R.B.C.*		Hb.*		Ht.*		M.C.V.*		M.C.Hb.*		M.C.Hb.C.*		%		Absolute		W.B.C.*	
		X	σ	X	σ	X	σ	X	σ	X	σ	X	σ	X	σ	X	σ	X	σ
4	4	2.815	0.299	8.60	.25	30.00	0.36	107.8	12.3	30.9	3.9	28.7	1.1	33.3	4.4	0.940	.173	2.15	0.42
5	3	2.405	0.250	8.34	.33	27.63	0.39	116.4	12.4	35.1	4.1	30.2	0.6	29.5	2.7	0.955	.422	3.15	1.54
6	4	3.415	0.372	8.34	.14	28.83	0.78	89.5	7.3	25.0	2.7	29.7	1.5	30.0	3.1	0.982	.164	4.12	1.65
7	4	2.895	0.501	8.64	.25	30.25	1.64	107.5	7.6	30.7	5.3	28.6	1.2	24.4	2.7	0.700	.100	3.11	0.33
8	4	2.900	0.126	8.31	.23	28.88	0.85	99.3	3.9	28.7	1.3	29.0	1.1	31.0	11.4	0.895	.310	3.64	0.89
9	4	3.490	0.465	9.06	.76	30.78	0.85	89.5	9.9	26.3	1.9	29.5	2.3	31.9	5.0	1.131	.280	3.69	0.44
10	8	3.710	0.113	8.90	.35	30.50	1.02	82.4	3.4	24.6	1.3	29.4	0.9	33.8	7.9	1.252	.275	3.63	0.75
11	11	3.445	0.177	7.99	.49	27.93	1.11	81.4	3.2	23.2	1.7	28.6	1.3	26.8	5.9	0.916	.184	3.61	0.86
12	8	3.585	0.150	7.61	.56	25.78	2.87	72.3	8.8	27.1	3.4	29.5	1.3	26.9	3.6	0.968	.152	4.28	0.81
13	5	4.135	0.122	8.46	.50	28.50	0.87	69.1	3.8	20.5	1.0	29.7	0.8	16.6	2.9	0.682	.141	4.81	0.46
14	10	4.485	0.421	9.19	.86	31.05	3.15	69.3	4.0	20.6	1.2	29.6	1.5	21.8	4.6	0.971	.201	4.40	1.99
15	12	4.000	0.403	7.78	.50	26.64	1.53	67.4	4.4	19.5	1.2	29.2	1.0	19.5	3.0	0.769	.121	3.58	0.89
16	12	3.855	0.374	7.73	.48	25.79	1.86	67.6	4.9	20.2	1.0	29.9	1.3	15.0	3.6	0.570	.157	4.44	0.89
17	15	4.230	0.197	8.08	.67	26.37	2.81	62.5	7.5	19.0	1.7	31.0	2.2	19.7	5.9	0.838	.244	3.56	0.69
18	6	4.230	0.155	7.22	.33	23.59	0.85	55.8	4.7	17.1	1.6	30.7	1.3	21.0	1.7	0.889	.088	4.14	1.13
19	11	4.185	0.332	7.13	.54	24.39	1.43	58.5	1.3	17.3	2.0	29.3	1.6	17.9	3.7	0.750	.131	5.48	1.53
20	4	4.875	0.351	8.74	2.24	28.00	5.22	57.0	7.1	17.7	3.5	30.8	2.2	22.2	8.8	1.053	.351	4.28	1.49
21	6	4.600	0.196	8.03	.18	28.21	1.80	61.7	2.9	17.5	0.9	29.5	1.4	23.6	2.7	1.076	.051	4.61	0.49
22	12	4.905	0.531	7.91	.59	27.40	3.07	56.0	5.4	16.2	2.9	29.0	1.7	26.3	6.8	1.180	.328	3.40	0.78
23	9	5.595	0.714	9.06	.49	32.08	1.73	58.1	5.3	16.4	4.0	28.2	0.7	22.9	5.7	1.313	.479	4.39	1.85
24	4	5.415	0.806	9.04	.78	32.31	2.77	61.6	14.1	17.2	4.0	28.0	1.1	22.8	2.8	1.223	.148	4.75	1.47

\*R.B.C. = Erythrocytes in millions per mm<sup>3</sup>; Hb. = hemoglobin in g per 100 cc blood; Ht. = % packed erythrocytes; M.C.V. = Mean corpuscular volume in cubic micra; M.C.Hb. = Mean corpuscular hemoglobin in gamma gamma; M.C.Hb.C. = Mean corpuscular hemoglobin concentration in %; Reticulocytes expressed in % erythrocytes and absolute in millions per mm<sup>3</sup> W.B.C. = Leucocytes in thousands per mm<sup>3</sup>; N = Number of rats.

flection maintained. A few isolated means of the other 8 blood values were statistically significant; the remainder were not. Thus, the great majority of the variations observed could have occurred by chance.

Comparison of the values of the 2 liver-injected rats with their litter mate control showed that the control value was located between the 2 corresponding values of the injected rats in 41%, 39%, and 42% of instances on the fourth, tenth and fifteenth days after injection respectively. This is approximately what should occur by chance. The blood values of the 30 injected rats between the twenty-fifth and thirtieth days continued the trends of the control group and were comparable to the litter control rats. Of the total of 1,368 determinations, only 0.95% were beyond the limits of  $X \pm 3\sigma$  of the control group.

Figures 1 and 2 present graphically the most useful criterion, the mean corpuscular volumes of the erythrocytes, in estimating the transition changes in the blood of the experimental groups as compared to the control group of young rats. None of the means are statistically significant.

Growth in both experimental groups of rats as judged by the weight and crown-tailbase length was comparable to control data.

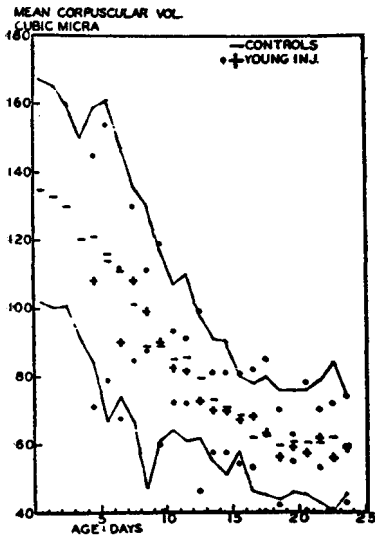


FIG. 1.

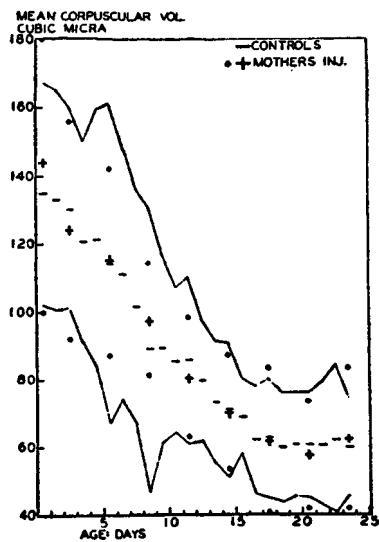


FIG. 2.

Figs. 1 and 2 illustrate the location of the means (+) and the  $\pm 3\sigma$  (•) of the mean corpuscular volumes of the erythrocytes of the 2 experimental groups of young rats at indicated ages after birth in relation to the mean (—)  $\pm 3\sigma$  (continuous line) of the control rats.

The lack of a marked acceleration of normoblastic erythropoiesis or of a lessened megaloblastic erythropoiesis is indicated especially by the not significantly different decline of the mean corpuscular volume, level of the reticulocytes, and increase of erythrocytes. Fitz-Hugh, Creskoff and Taylor<sup>8</sup> indicated the ineffectiveness of daily injections of liver extract in changing the reticulocyte count of 14 nursing rats. The injection of liver extract into the fetuses of 2 pregnant rats was likewise ineffective. Wintrobe, *et al.*,<sup>9</sup> found that liver extract injected into 3 pregnant rabbits produced no effect on the erythrocytes of fetuses extracted before term. Also, the injection of 0.15 cc of liver extract into the placentas of rabbit fetuses in one horn of the uterus did not alter the blood picture of those fetuses in comparison with the fetuses in the uninjected horn. These workers reported that extracts of fetal pig livers and of the placenta, even in the last third of pregnancy, were inactive. Wigodsky, *et al.*,<sup>10</sup> however, found fetal calf livers anti-anemically active. Wigodsky and Ivy<sup>11</sup> injected liver extract into pregnant rats from 2 to 14 days before delivery. They observed no significant differences between the values of the erythrocytes, hemoglobin, hematocrit, mean corpuscular volume, or the mean red cell diameter of new born rats from normal and antenatally injected mothers. Their data indicate variations similar to those encountered above.

Briese and Higgins<sup>12</sup> have reported that the addition of ventriculin to the diet of pregnant rats resulted in a decrease of the mean diameter of the erythrocytes of the new born rats together with a shift of the Price-Jones curve to the left as measured from dry smears. The variability in the erythrocyte counts of normal new born led them to suggest that variations in the maternal stores of the anti-anemic principle may occur. Stasney and Higgins<sup>13</sup> have reported that the daily intraperitoneal injection of neutralized normal human gastric juice into pregnant rats for 12 days preceding parturition resulted in a reduction of the diameters and corpuscular volumes of the erythrocytes of the new born. Heated gastric juice

<sup>8</sup> Fitz-Hugh, T., Jr., Creskoff, A. J., and Taylor, H. B., *J. Clin. Invest., Proc.*, 1936, **15**, 468.

<sup>9</sup> Wintrobe, M. M., Kinsey, R. E., Blount, R. C., and Trager, W., *Am. J. Med. Sci.*, 1937, **103**, 449.

<sup>10</sup> Wigodsky, H. S., Richter, O., and Ivy, A. C., *Am. J. Physiol., Proc.*, 1938, **123**, 215.

<sup>11</sup> Wigodsky, H. S., and Ivy, A. C., *Proc. Soc. Exp. Biol. and Med.*, 1938, **38**, 787.

<sup>12</sup> Briese, E., and Higgins, G. M., *Anat. Rec.*, 1939, **73**, 105.

<sup>13</sup> Stasney, J., and Higgins, G. M., *Proc. Staff Meetings Mayo Clinic*, 1937, **12**, 490.

produced no effect. Similar effects were obtained by Stasney, Higgins, and Mann<sup>14</sup> using the gastric juice obtained from a fundic pouch of a pig. Briese<sup>15</sup> reported that the blood picture of the new born of rats from mothers rendered anemic by daily inhalations of CCl<sub>4</sub> during pregnancy was more macrocytic than the blood of young from normal mothers.

The lack of significant changes probably are not due to administration of insufficient amounts nor to the mode of administration of liver extract. Connery and Goldwater<sup>16</sup> and Riddle and Sturgis<sup>17</sup> have shown that single massive doses are effective in inducing remission in human pernicious anemia. It is interesting to observe also that the liver preparation did not produce a reduction in erythrocytes or hemoglobin. This effect of liver has been reported by Marshall<sup>18</sup> after injection of liver into rats made polycythemic by cobalt. Adlersberg and Leiner<sup>19</sup> reported that anemia may be produced in normal rabbits by the administration of liver preparations both orally and parenterally; a powdered liver preparation given orally to dogs also produced an anemia.

Assuming that the rat requires the anti-pernicious anemia principle, the lack of effect of the administered liver extract in the young rats is similar to that observed in the human macrocytic hyperchromic anemia patient previously given optimal amounts of liver extract. It would appear that in the presence of adequate amounts of anti-anemia substance some other factor becomes the limiting agent in the mechanism of blood production.

*Summary.* 1. The transition from megaloblastic to normoblastic erythropoiesis appears to be reflected in data on the blood cell values of normal rats from birth to 24 days of age. 2. Blood cell values of young whose mothers were injected with liver extract before and during pregnancy were found to be not significantly different from control values at comparable ages after birth. 3. Blood cell values of young rats directly injected with liver extract up to the fourteenth day after birth were found not to be significantly different from control values at comparable ages after birth. 4. The young rat's reaction to the presence of excess anti-anemic principle resembles that of the human macrocytic hyperchromic anemia patient maintained on adequate amounts of liver extract.

<sup>14</sup> Stasney, J., Higgins, G. M., and Mann, F. C., *Proc. Staff Meetings Mayo Clinic*, 1937, **12**, 699.

<sup>15</sup> Briese, E., *Am. J. Med. Sci.*, 1938, **195**, 787.

<sup>16</sup> Connery, J. E., and Goldwater, L. J., *J. Lab. and Clin. Med.*, 1932, **17**, 1016.

<sup>17</sup> Riddle, M. C., and Sturgis, C. C., *Am. J. Med. Sci.*, 1930, **180**, 1.

<sup>18</sup> Marshall, L. H., *Am. J. Physiol.*, 1936, **114**, 194.

<sup>19</sup> Adlersberg, D., and Leiner, G., *Z. f. d. ges. exper. Med.*, 1936, **98**, 398.